FIBER OPTIC-BASED BIOSENSOR FOR ENVIRONMENTAL APPLICATIONS

Lisa C. Shriver-Lake and Frances S. Ligler
Center for Bio/Molecular Science and Engineering,
Naval Research Laboratory,
Washington, DC 20375-5348
(202)404-6045
(202)767-9594
lcs@cbmse.nrl.navy.mil

ABSTRACT

The NRL fiber optic biosensor is a device which measures the formation of a fluorescent complex at the surface of an optical fiber. Antibodies provide a mechanism for recognizing an analyte of interest and immobilizing a fluorescent complex onto the fiber surface. The fiber optic biosensor is fast, sensitive, and it permits analysis of hazardous materials remote from the instrumentation. Antibodies coated on the fiber are stable for up to two years of storage prior to use. A portable fiber optic biosensor has been developed which is able to monitor four fiber probes simultaneously. Quadruplicates or 4 separate samples can be analyzed with the multiple fiber system. The biosensor has been used to measure concentration of toxins, proteins, small molecules in the parts per billion (μg/L) range in under 10 minutes. The fiber optic biosensor is currently being applied for detection of explosives, pathogens, and toxic materials which pollute the environment. On-site TNT analysis of explosive contaminated groundwater and leachate samples has been demonstrated.

INTRODUCTION

A biosensor is a detection system which exploits the sensitivity and selectivity of a biomolecule (i.e. an antibody) for analyte recognition and incorporates that biomolecule into an optoelectronic device for signal transduction (1-3). The NRL biosensor employs immobilized antibodies as the recognition component on the exposed fiber optic core. Upon binding of the analyte of interest, a fluorescence signal is generated at the surface of the optical fiber and the signal is transmitted back to a photodetector. To limit sample preparation and assay washing steps, signal generation occurs within a 100 nm area surrounding the fiber core referred to as the evanescent wave. Quantitative results can be determined based on the fluorescent signal levels. The fiber-optic biosensor is described here in terms of the optical components, chemistry for protein immobilization, and assay development.

OPTICAL DEVICE

Initial assay development was performed on a laboratory breadboard device (4). Recently, a portable fiber optic sensor was developed in collaboration with Research International (Woodinville, WA). This device, the Analyte 2000, is small (6.5" x 4.5" x 3.5"), lightweight (2.5 lbs.), portable, operates on either battery or 110V, and is able to monitor four fiber probes simultaneously (5,6). It employs 635 nm diode lasers for excitation and photodiodes to collect the returning fluorescence signal. A jumper cable of variable length connects the device to the antibody-coated optical probe.

The distal or "business" end of the fiber probe receives intensive preparation. The 600 μm fibers are decladded over the last 10 cm to expose the fiber core which forms the sensing region. To prevent signal loss, a method for propagating the fluorescent signal nearer to the center of the fiber by

tapering the declad region with hydrofluoric acid has been developed (2). The geometry that yields the most power in the evanescent wave, the least excitation of bulk fluorescence, and the best propagation of emitted light is a combination taper (7,8).

ANTIBODY IMMOBILIZATION CHEMISTRY

Once the fiber is tapered, the antibodies responsible for detection are immobilized on the unclad surface of the fiber probe. As described elsewhere (9,10), the fibers are cleaned and coated with a thiol-terminal silane. A heterobifunctional crosslinker, which reacts at one end with thiol groups from the silane and at the other end with terminal amino groups on the antibody, is used to attach antibodies covalently to the fiber surface. Antibodies are routinely immobilized at 2 ng/mm^2 (2) with a capacity to bind large protein antigens at a ratio of 1 antigen per 4 antibodies. Antibodies immobilized using this procedure, maintain antigen binding capability after storage of almost 2 years.

ASSAYS

Three types of immunoassays are being developed for use with the fiber optic-based biosensor for environmental applications. These assays include those specific for small molecules, proteins, and bacteria (11). In all of these assays, signal generation begins immediately upon introduction of the probe into the solution containing the fluorescent reagent. Fibers coated with irrelevant antibodies are used to control for false positive signals.

The main environmental assay with this biosensor is a competitive immunoassay which is used for the detection of small molecules such as explosives. In this assay, the analyte of interest (i.e., TNT) competes with a fluorescently-labeled analog for binding sites on the immobilized antibody. In this assay, an inhibition of the fluorescent signal indicates the presence of the analyte. The percent of inhibition is proportional to the concentration of the analyte of interest. The explosive TNT can be detected at low ppb levels with this system (12,13). With the TNT assay, the fiber probe can be used multiple times by removing bound analyte with 50% ethanol. This permits multiple samples and the reference samples without TNT to be analyzed on the same fiber. To demonstrate the performance of the fiber optic biosensor with environmental samples, detection and quantification of TNT in groundwater and leachate samples were performed at Umatilla Army Depot (Hermiston, OR) and Naval Submarine Base Bangor (Bangor, WA). The results from the fiber optic biosensor correlated well with splits analyzed via U.S. EPA SW-846 Method 8330 in a laboratory.

For detection of larger molecules, such as proteins and bacteria, a sandwich immunoassay was developed where the analyte of interest is bound between the immobilized antibody on the fiber and fluorescently-labeled antibody in solution (11,14). The increase in the fluorescent signal is proportional to the concentration of the analyte. Sensitivities of 1-10 μg/L have been obtained for several protein toxins including *Staphylococcal enterotoxin B*, ricin toxin, F1 antigen from *Yersinia pestis,* and endotoxin (6, 11,15). Several successful on-site analyses for the larger molecules have been performed.

A direct immunoassay can also be employed to detect bacteria (16). Cells are nonspecifically stained with a fluorescent dye. The cells are then exposed to the antibody-coated fiber optic probe. The cells of interest are bound by the immobilized antibody onto the fiber generating a fluorescent signal. As with the sandwich assay, the increase in fluorescent signal is proportional to number of cells of interest. The un-bound cells are located outside the evanescent wave and do not generate a signal. Detection of 3000 cells/ml have been achieved.

CONCLUSION

NRL has developed a portable fiber optic biosensor which can use long fibers for analysis remote from the optical components. The fiber optic probes are configured for homogeneous assays using the evanescent wave and the probe's geometry to significantly improve the signal transmission back up the fiber. Chemistry for immobilizing protein onto the fiber that successfully immobilized a high density of functional molecules has been developed. Antibodies can be immobilized on the fiber up to two years before use and remain functional. A wide variety of environmental assays has been developed which transduces a binding event into a fluorescent signal. Small and large molecules and pathogenic organisms have been detected in less than 10 minute. This biosensor has successfully analyzed samples on-site for TNT, protein toxins, and bacteria.

NOTES AND ACKNOWLEDGMENTS

Five patents (7,8,10,15,16) have been filed covering this technology and are available for license. The work would not have been possible without support from the Office of Naval Research (ONR), the U.S. Naval Medical R&D Command, the Environmental Security Certification Program (ESTCP) and Advanced Research Program Agency (ARPA). The authors particularly thank Brian Donner, Mark Pease, Joel Golden, George Anderson, Daya Wijesuria, Robert Ogert, Richard Thompson, and Carl Villaruel for their critical scientific contributions. The views expressed here are that authors own and do not reflect policy of the U.S. Navy, Department of Defense or United States Government.

REFERENCES

1. Buck, R.P. et.al., eds; Biosensor Technology, Marcel Dekker, Inc. NY, 1990, 419 pp.

2. D.L. Wise, L.B. Wingard, Jr., eds; Fiber Optic Biosensors, Humana Press, NJ, 1991, 370 pp.

3. Wolfbeis, O.S., ed.; Fiber Optic Chemical Sensors and Biosensors, CRC Press, Inc. Boca Raton, FL, 1991, Vol. I, 385 pp, Vol 2, 341 pp.

4. J.P. Golden, L.C. Shriver-Lake, G.P. Anderson, R.B. Thompson, and F.S. Ligler, An evanescent wave biosensor using tapered fiber optic probes. Optical Engineering, 31(7), 1458-1462, 1992.

5. E.W. Saaski, M. Bizak, and J. Yeatts. Multimode evanescent wave-based sensors. SPIE Procceedings, 2574, 56, 1995

6. L.A. Tempelman, K.D. King, G.P. Anderson, and F.S. Ligler. Quantitating *Staphylococcal enterotoxin B* in diverse media using a portable fiber optic biosensor. Anal. Biochem., 223, 50-57, 1996.

7. R. Thompson and C. Villarruel, Waveguide-binding sensor for use with assays US Patent #5,061,857, issued 10/29/91.

8. Anderson, G.P. and J.P. Golden, Mode-matched combination taper fiber optic probe. U.S. Patent 5,430,813, issued 7/4/95.

9. Bhatia, S.K., L.C. Shriver-Lake, K.J. Prior, et al; Use of thiol-terminal silanes and heterobifunctional crosslinkers for immobilization of antibodies on silica surfaces. Anal. Biochem, 178, 408-413, 1989.

10. F.S. Ligler (misspelled Eigler by patent office), J. Calvert, J. Georger, L. Shriver-Lake, S. Bhatia, and R. Bredehorst, Immobilization of active agents on substrates with a silane and heterobifunctional crosslinker U.S. Patent No. 5,077,210. Issued 12/31/91.

11. F.S. Ligler, J.P. Golden, L.C. Shriver-Lake, R.A. Ogert, D. Wijesuria, and G.P. Anderson. Fiber optic biosensor for the detection of hazardous materials. Immunomethods, 3(2), 122-127, 1993.

12. L.C. Shriver-Lake, K.A. Breslin, J.P. Golden, L. Judd, J. Choi, and F.S. Ligler. A fiber optic biosensor for the detection of TNT. SPIE, 2367, 52-58, 1994.

13. L.C. Shriver-Lake, K.A. Breslin, P.T. Charles, D.W. Conrad, J.P. Golden, and F.S. Ligler. Detection of TNT in water using an evanescent wave fiber optic biosensor. Anal Chem, 67(14), 2431-2435, 1995.

14. G.P. Anderson, J.P. Golden, L.K. Cao, D. Wijesuriya, L.C. Shriver-Lake, and F.S. Ligler. Development of an evanescent wave fiber optic biosensor. IEEE Eng. Med. Biol., 13, 358-363, 1994.

15. F.S. Ligler and E.A. James, Rapid assay for detection of endotoxins, U.S. Patent Application # 08/422103, submitted 4/95.

16. F.S. Ligler, L.C. Shriver-Lake, and D.C. Wijesuriya, Optical immunoassay for microbial analytes using non-specific dyes, International Appl. # PCT/USA/08752, U.S. Patent No. 5,496,700 issued March 5, 1996.